

Mechanisms for Ca²⁺-dependent permeability transition in mitochondria

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In a recent study in cells lacking an assembled F-ATP synthase the conclusion was reached that this enzyme cannot form the mitochondrial permeability transition pore (PTP) (1). As in previous studies (2, 3) the key argument is that mitochondria still undergo cyclosporin A (CsA)-sensitive swelling and Ca²⁺-induced Ca²⁺ release (1–3). The PTP (or mitochondrial megachannel, MMC) is an inner membrane channel activated by matrix Ca²⁺ and inhibited by CsA through cyclophilin (CyP) D, a matrix peptidyl-prolyl *cis*–*trans* isomerase. The PTP can reach conductances up to 1.2 to 1.5 nS but is rich in smaller subconductance states (4). Based on the effects of the adenine nucleotide translocase (ANT) inhibitors carboxyatractylate and bongkrekate (BKA) (PTP activation and inhibition, respectively) the first candidate for PTP formation was the ANT, which forms BKA-sensitive, Ca²⁺-activated channels with conductance up to 0.6 nS affected by CyP in a CsA-sensitive manner (5).

The ANT hypothesis was questioned because a PT could be detected in liver mitochondria after ablation of the genes encoding for ANT1 and ANT2, isoforms expressed in mouse liver (6), but this hypothesis should be reevaluated. Indeed, ANT1/ANT2-null mice overexpress ANT4 in the liver (7). Although ANT1/ANT2-null mitochondria did not show any appreciable respiratory stimulation with ADP (6), it is possible that ANT4 could have contributed to Ca²⁺-dependent permeabilization (7). At any rate, generation of ANT1/ANT2/ANT4 triple-knockout (KO) mice allowed readdressing the role of ANT in the PT with some interesting results. Triple-KO mitochondria became more resistant to the

Ca²⁺-dependent PT yet underwent full permeabilization to sucrose, indicating that the PTP still exists in the absence of any ANT isoforms, and treatment with CsA or ablation of CyPD made mitochondria refractory to the PT (7), indicating that the channels mediating permeabilization are all activated by CyPD and inhibited by CsA.

Mitochondria in cells lacking an assembled F-ATP synthase 1) displayed a slower swelling rate and extent both in KCl- and sucrose-based media, 2) initiated the swelling response in sucrose after a lag phase of about 30 s from Ca²⁺ accumulation, at variance with wild-type mitochondria where swelling immediately followed Ca²⁺ uptake, and 3) markedly increased their Ca²⁺ retention capacity after treatment with both CsA and BKA (1). Inhibition by BKA, which does not affect the channel activity of F-ATP synthase (8), suggests that the actual permeabilization pathway in mitochondria that do not assemble a functional F-ATP synthase is provided by the ANT. This is also consistent with observations in cells where subunit c of F-ATP synthase had been ablated, resulting in lack of assembly of F-ATP synthase (2). Mitochondria in these cells no longer display PTP/MMC channel activity but possess a smaller channel of about 0.3 nS that is inhibited by BKA (9). Taken together, these findings suggest that mitochondria have at least two pathways for permeabilization: 1) the F-ATP synthase, which is inhibited by CsA but not BKA (8, 9), and 2) the ANT, which is inhibited by both CsA and BKA (9). Thus, F-ATP synthase remains an excellent candidate for the high-conductance PTP (8).

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